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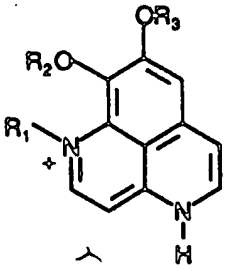
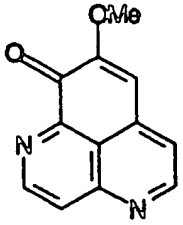
(57) Abstract

The present invention provides a use of a compound of formula (I), wherein: R_1 is methyl or hydrogen, R_2 is methyl, acetyl, benzoyl, or hydrogen, and R_3 is methyl, acetyl, benzoyl or hydrogen; a compound of formula (II), or a pharmaceutically acceptable salt thereof, for the treatment of cellular signalling-mediated, especially PKC-mediated, disease states such as cancer, cardiovascular, renal, and central nervous system disorders, inflammation, immunosuppression and septic shock; a method of treatment thereof; pharmaceutical compositions comprising said compound; and a compound of formula (I) wherein R_1 is methyl, R_2 is hydrogen, and R_3 is methyl, acetyl, or benzoyl.



(I)



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AAPTAMINES AND METHOD OF USE THEREOF

FIELD OF THE INVENTION

The present invention relates to the treatment and prevention of disease states mediated by cellular signalling, especially wherein protein kinase C inhibition is indicated. The present invention relates to the treatment of cancer, cardiovascular, renal, and central nervous system disorders, inflammation, immunosuppression and septic shock by the use of marine alkaloids of the aaptamine class for the treatment of such cellular signalling-mediated, especially protein kinase C-mediated, disease states.

BACKGROUND OF THE INVENTION

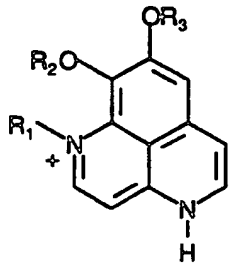
Many disease states are known to be mediated by cellular signalling. For instance, protein kinase C (hereinafter "PKC") is a calcium and phospholipid activated enzyme that plays a significant role in mediating the effects of a host of hormones, neurotransmitters, growth factors, antigens and inflammatory mediators (Nishizuka, Y. (1988) Nature, 334:661). When these extracellular agents bind to their specific cell surface receptors, they stimulate the hydrolysis of phosphatidylinositol, phosphatidyl choline or phosphatidylethanolamine, resulting in the accumulation of diacylglycerol which, in turn, activates PKC. This activation of PKC causes specific cellular substrates to be phosphorylated, resulting in the regulation of cellular processes which are closely linked to the physiological control of contractile, secretory and proliferative processes (Nishizuka, Y. (1984) Nature, 308:693). Reported examples of physiological responses induced by the system in which PKC participates include proto-oncogene activation (Nishizuka, Y. (1986) Science, 233, 305-312), serotonin release from platelets (Kaibuchi, et al. (1982) Cell Calcium, 3:323; Kaibuchi, et al. (1985) J. Biol. Chem., 258:6701), lysosomal enzyme release and superoxide generation from neutrophils (Kajikawa, et al. (1983) Biochem. Biophys. Res. Commun., 116:743; Sehau, et al. (1983) Biochem. Biophys. Acta, 762:420), histamine release from mast cells (Kata Kami, et al. (1982) Biochem. Biophys. Res. Commun., 121:573), secretion of aldosterone from adrenal glomerulus (Kojima, et al. (1983) Biochem. Biophys. Res. Commun., 116:555), and contraction of vascular smooth muscle (Rasmussen, et al. (1984) Biochem. Biophys. Res. Commun., 122:776).

Thus, it has been demonstrated that cellular signalling, for instance by PKC, is a key component of many important physiological responses *in vivo*. Therefore, an inhibitor of cellular signalling would be expected to be useful in the treatment of a broad variety of disease states known to be mediated by cellular signalling. In particular, an inhibitor of PKC would be expected to be useful in the treatment of PKC-mediated disease states including without limitation cancer, cardiovascular, renal, and central nervous system disorders, inflammation, immunosuppression, septic shock and other PKC-mediated disease states.

Surprisingly, we have recently found that marine alkaloids of the aaptamine class, several of which have been previously described (Nakamura, et al. (1987) J. Chem. Soc. Perkin Trans. I, 173-176) as alpha-adrenoceptor blocking agents (Ohizumi et al. (1984) J. Pharma. Pharmacol. 36:785), also function as inhibitors of cellular signalling, in particular as PKC inhibitors, and hence have utility in the treatment of disease states wherein inhibition of cellular signalling, in particular PKC inhibition, is indicated for a curative or ameliorative effect.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides a method of treatment of cellular signalling-mediated, especially PKC-mediated, disease states in mammals comprising administering to a mammal in need of such treatment an effective amount of a compound of Formula (I):



(I)

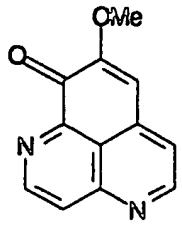
wherein:

R_1 is methyl or hydrogen;

R_2 is methyl, acetyl, benzoyl, or hydrogen; and

R_3 is methyl, acetyl, benzoyl or hydrogen;

or Formula (II):



(II)

or a pharmaceutically acceptable salt thereof.

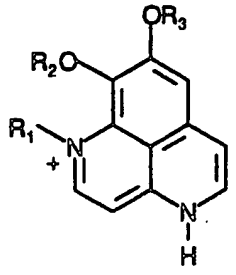
In another aspect, the present invention provides a use for such a compound of Formula (I), a compound of Formula (II), or a pharmaceutically acceptable salt thereof for treatment of cellular signalling-mediated, especially PKC-mediated, disease states in mammals.

In yet another aspect, the present invention provides pharmaceutical compositions comprising a compound of Formula (I), a compound of Formula (II), or a pharmaceutically acceptable salt thereof for the treatment of conditions wherein inhibition of cellular signalling is indicated, especially for the treatment of conditions where PKC inhibition is indicated, for example, in the treatment of cancer, cardiovascular disorders, renal disorders; inflammation, central nervous system disorders, immunosuppression and septic shock.

In still another aspect, the present invention provides a compound of Formula (I) wherein: R_1 is methyl; R_2 is hydrogen; and R_3 is methyl, acetyl, or benzoyl; preferably a compound wherein:R_1 is methyl, R_2 is hydrogen, and R_3 is benzoyl, preferably bromobenzoyl, most preferably 4-bromobenzoyl; or wherein: R_1 is methyl, R_2 is hydrogen, and R_3 is methyl.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a method for treating disease states in mammals, including humans, wherein inhibition of cellular signalling is indicated, especially wherein PKC inhibition is indicated. The method comprises administration to a mammal, preferably a human, in need thereof, at least one of a class of cellular signalling, preferably PKC, inhibitors of Formula (I):



(I)

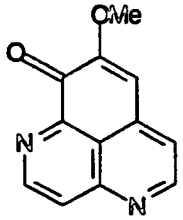
wherein:

R_1 is methyl or hydrogen;

R_2 is methyl, acetyl, benzoyl, or hydrogen; and

R_3 is methyl, acetyl, benzoyl or hydrogen;

or of Formula (II):



(II)

or a pharmaceutically acceptable salt thereof.

The present invention also provides a use for such a compound of Formula (I), a compound of Formula (II), or a pharmaceutically acceptable salt thereof for treatment of cellular signalling-mediated, especially PKC-mediated, disease states in mammals.

The present invention additionally provides pharmaceutical compositions comprising a compound of Formula (I), a compound of Formula (II), or a pharmaceutically acceptable salt thereof for the treatment of conditions wherein inhibition of cellular signalling, especially PKC, is indicated.

In addition, the present invention provides a novel compound of Formula (I) wherein: R_1 is methyl; R_2 is hydrogen; and R_3 is methyl, acetyl, or benzoyl; preferably a compound wherein: R_1 is methyl, R_2 is hydrogen, and R_3 is benzoyl, preferably bromobenzoyl, most preferably 4-bromobenzoyl; or wherein: R_1 is methyl, R_2 is hydrogen, and R_3 is methyl.

The cellular signalling, preferably PKC, inhibitors of Formula (I) and Formula (II) of the present invention are useful for the treatment of cellular signalling-mediated, especially PKC-mediated, disease states including, without limitation, cancer, preferably as adjuvant therapy for use with antineoplastic

compounds to ameliorate or prevent multiple drug resistance (MDR) of the target neoplastic cells to such antineoplastic compounds; cardiovascular diseases, that is heart and circulatory diseases such as thrombosis, atherosclerosis, arteriosclerosis, ischemia, reperfusion injury, and hypertension, preferably hypertension; immunosuppressive and inflammatory disorders, such as asthma, rheumatoid arthritis, psoriasis, inflammatory bowel disease, and acquired immune deficiency syndrome (AIDS), preferably AIDS and psoriasis; central nervous system diseases, such as stroke and trauma; septic shock based on PKC activation and ischemia-induced renal failure. The method of treatment of the present invention concerns the use of compounds of Formula (I) and Formula (II) as cellular signalling, preferably PKC, inhibitors in the treatment of such cellular signalling-mediated, especially PKC-mediated, disease states.

The terms "benzoyl" and "benzoyl esters" are understood to include both the unsubstituted benzoyl group as well as the benzoyl group substituted with commonly known substituents at any position on the ring, preferably a halogen, more preferably chloro, bromo, or iodo, yet more preferably bromo, most preferably 4-bromo. The term "cellular signalling", also known in the art as "signal transduction", means the interactions of a molecule at the cell surface, which interactions are communicated to the interior of the cell, usually the nucleus, via biochemical pathways, ultimately resulting in a physiological response.

The compounds of Formula (I) wherein R_3 is methyl have been isolated from the marine sponge, *Aaptos aaptos* (Nakamura, et al. (1987) J. Chem. Soc. Perkin Trans I, 173-176). Iso-aaptamine, that is, the compound of Formula (I) wherein R_1 and R_3 are methyl and R_2 is H, is conveniently isolated from the marine sponge, *Aaptos aaptos*, as follows. The sponge is frozen upon collection. The frozen sponge is then fragmented and extracted with ethyl acetate and methanol. The desired compound is readily isolated by thick layer chromatography on silica gel. The acetyl and benzoyl esters of iso-aaptamine may be conveniently prepared using methods well-known in the art.

A pharmaceutical composition of the present invention comprises a compound of Formula (I) or Formula (II) and an appropriate pharmaceutical carrier, diluent, or excipient. Such appropriate pharmaceutical carriers, diluents, or excipients may be either solid or liquid. Such a pharmaceutical composition may be parenterally, rectally, topically, transdermally or orally administered, preferably

orally. Pharmaceutical forms include, but are not limited to, syrups, suspensions or emulsions, tablets, capsules and lozenges.

A liquid formulation will generally consist of a suspension or solution of the compound or pharmaceutically acceptable salt thereof in a suitable liquid carrier. Suitable liquid carriers include, but are not limited to, ethanol, glycerin, non-aqueous solvents such as polyethylene glycol, oils, or water with a suspending agent, preservatives, flavorings, or coloring agents, or any suitable combination thereof.

A composition in the form of a tablet can be prepared using any suitable pharmaceutical carrier routinely used for preparing solid formulations. Examples of such carriers include, but are not limited to, magnesium stearate, starch, lactose, sucrose and cellulose.

A composition in the form of a capsule can be prepared using routine encapsulation procedures. For example, pellets, granules or powder containing a compound of Formula (I) or Formula (II) can be prepared using standard carriers and then filled into a hard gelatin capsule. Alternatively, a dispersion or suspension can be prepared using any suitable pharmaceutical carrier(s) and the dispersion or suspension is then filled into a soft gelatin capsule. Suitable pharmaceutical carriers include aqueous gums, celluloses, silicates and oils.

A composition for parenteral administration can be formulated as a solution or suspension. Said solution or suspension will generally consist of a compound of Formula (I) or Formula (II) in a sterile aqueous carrier or parenterally acceptable oil. Examples of parenterally acceptable oils include, but are not limited to, polyethylene glycol, polyvinyl pyrrolidone, lecithin, arachis oils and sesame oil. Alternatively, the solution can be lyophilized and then reconstituted with a suitable solvent just prior to administration.

The pharmaceutical preparations are made following conventional techniques of a pharmaceutical chemist and involve mixing, granulating, and comtransdermal, or topical products.

Preferably the composition is in unit dose form. Each dosage unit for parenteral or oral administration contains preferably from 100 mg to 1000 mg of a compound of Formula (I) or Formula (II) or a pharmaceutically acceptable salt thereof.

The daily dosage regimen for a subject in need of PKC inhibition may be, pressing, when necessary, for tablet forms, or mixing, filling and dissolving the

ingredients, as appropriate, to give the desired oral, parenteral, rectal, for example, an intravenous, subcutaneous, or intramuscular dose of between 100 mg and 1000 mg of a compound of Formula (I) or Formula (II) or a pharmaceutically acceptable salt thereof, the compound being administered 1 to 4 times per day. Suitable compounds will be administered for a period of continuous therapy. Dosages for oral administration may be higher.

No unacceptable toxicological effects are expected when compounds of the present invention are administered in accordance with the present invention.

BIOLOGICAL DATA

The following tests were performed using the compound of Formula (I) wherein R_1 is methyl, R_2 and R_4 are hydrogen, and R_3 is methyl, designated as iso-aaptamine.

1. *In Vitro* Enzyme Assays:

A. Rat Brain Screening Assay

Purification of Protein Kinase C from Rat Brain

Protein kinase C is purified from rat brain following the procedure of Walton et al. (Walton, G.H., Bertics, P.J., Hudson, L.G., Vedvick, T.S., and Gill, G.N., Anal. Biochem. 161:425-437 (1987)) and Woodget and Hunter (Woodget, J.R. and Hunter, T., J. Biol. Chem. 268:4836-4843 (1987)) with the following modifications. Ammonium sulfate precipitation is performed twice (first time to 33% saturation, and the second to 70%). After centrifugation, pellets are resuspended and desalted using a G-100 column. Peak fractions are pooled, brought to a final concentration of 16% in glycerol and 0.01% Triton X-100, and frozen in small aliquots.

Screening for Protein Kinase C Inhibitors

A high throughput screening assay utilizing 96-well microtiter plates has been developed to identify potential inhibitors of protein kinase C. The incubation volume in each well is 50 microliters containing 10 mM Tris, pH 7.5, 1.1 mM $CaCl_2$; 10 mM $MgCl_2$; 1.0 mM EGTA, 40 micrograms/ml phosphatidyl serine, 1 microgram/ml Diolein; and 100 micrograms/ml histone. The reaction is initiated by addition of 0.5 microcuries of γ-^{32}P-ATP (10 micromolar final concentration) subsequent to addition of the various concentrations of test compounds or extracts. The reaction is stopped after 10 minutes at 37°C by spotting 25 microliters of the reaction mixture onto Whatman P81 paper squares using a multichannel pipettor. The squares are washed extensively in 0.5% phosphoric acid, dried with acetone, and assayed for radioactivity by liquid scintillation spectrometry. The concentrations of ATP, histone, and phosphatidyl serine used in the assay permit identification of inhibitors of both catalytic and regulatory sites of protein kinase C.

Results

In the above assay, the IC_{50} of iso-aaptamine for inhibition of PKC was about 100 to about 300 nM.

2. *In Vitro* Cellular and Tissue Assays:

A. Inhibitory Effect on PKC-mediated Differentiation of Human U937 Monocyte Cells to Macrophages Assay

Phorbol esters, which activate PKC in cells, induce human U937 monocytic cells to differentiate to macrophages. This assay measures amount of differentiation by following loss of undifferentiated monocyte cells from the suspension and increased adherence of macrophage cells to plastic. Human U937 monocytes in RPMI1640 media, supplemented with 10% heat inactivated fetal calf serum, 100 micrograms/ml penicillin and 100 micrograms/ml streptomycin, were incubated at 37°C in the presence of various concentrations of test compounds for 0, 1, 6 and 24 hours. The percentage of differentiation of cells was measured either by labeling cells with ^{14}C-thymidine in the DNA and determining the ratio of radioactivity attached to the plastic versus the sum of the radioactivity in the supernatant plus that radioactivity attached to the plastic or counting and comparing the number of cells in the supernatant versus the number of cells scraped from the plastic.

Results

In the above assay, the IC_{50} of iso-aaptamine for inhibition of PKC-mediated human monocytic cell differentiation was about 200 nM.

B. Toxicity Toward Cultured Cells Assay

Toxicity is determined by standard assays of growth inhibition following 72 hours of continuous exposure to compounds of the present invention. Microtiter plates are seeded with 2×10^3 (200 microliters) cells per well and allowed to attach overnight. The following day, the medium (EMEM containing 10% fetal bovine serum and antibiotic/antimycotic) is removed and fresh medium (180 microliters) is added. Test compounds are diluted into fresh media and 20 microliters is added to each well. After the compound is exposed to the cells for 3 days, 50 microliter of XTT/PMS solution is added. The XTT/PMS solution must be prepared fresh before using. The XTT solution is prepared in the following manner. Eight milligrams of XTT (Sigma X-4251) is dissolved in 100 microliters DMSO. This solution is then added to 3.9 ml of PBS without cations (Cf = 2 milligrams/ml). A stock solution of PMS (Phenazine methosulfate, Sigma P9625) is prepared by dissolving 10 mg of PMS in 3.3 mL of PBS without cations (Cf = 3 milligrams/ml). Twenty microliters of the PMS stock solution is then added to the XTT solution to form the XTT/PMS

solution. The plates are incubated at 37ºC for 90 minutes or until the $OD_{450} \geq 1.0$. The plate is blanked on wells without cells containing only 200 microliters of media and 50 microliters of XTT/PMS.

Results

In the above assay, toxicity of iso-aaptamine toward the cultured cells was observed at about 45 μM.

C. Reversal of MDR Phenotype Assay

Chinese hamster ovary cells (CHO) (line CH^RC5 -- a colchicine-resistant, PGp-overproducing, MDR cell line) were compared with the wild-type, drug-sensitive parental line with respect to sensitivity to killing by topotecan and production of topoisomerase I-mediated DNA strand breakage (topotecan produces topoisomerase I-linked DNA strand breaks in cells and kills them by converting the breaks into lethal lesions when the cells attempt to utilize the DNA as a template). The MDR cells were 10-15 fold resistant to killing by topotecan (in contrast, they are about 100-300 fold resistant to killing by vinblastine, a classical MDR drug) and sustained fewer DNA strand breaks when incubated with topotecan than did the parental cells. In the MDR cells, the production of topoisomerase I-linked breaks by topotecan is diminished because topotecan is effluxed from the cells by Pgp. Treatment of MDR cell cultures (CH^RC5) with active PKC inhibitors for approximately 2 hr before further incubation for one hr with topotecan has been found to increase the yield of DNA damage (consisent with enhanced drug accumulation and reduced drug efflux) was produced by 1 μM isoaaptamine, present 2 hr prior to and during the 1 hr topotecan treatment. The far less potent, realted compound aaptamine was virtually inactive in this assay even at ten times this concentration.

1. Cell Cultures. CHO cell lines AuxB1 (wild type, parental) and CH^RC5 (colchicine-resistant, Pgp-overexpressing, MDR progeny line) were kindly provided by Dr. Victor Ling, Ontario Cancer Center, Toronto, Ontario, Canada. The selection of CH^RC5 has been described Ling,V. et al. (1974) J. Cell Physiol. *83*: 103-116. Cells were maintained routinely in humidified 5% CO_2 incubators as monolayer cultures in α-MEM growth medium containing 10%fetal bovine serum were purchased from GIBCO. Cells were subcultured regularly using standard trypsinization procedures.

Multiple drug-resistant sublines of P388 leukemia were obtained originally from the National Cancer Institute tumor repository at Frederick Cancer Research Center, Frederick, MD. Cells were maintained by serial intreperitoneal (ip) implantation into DBA/2 mice, as described previously.

Geran, et al. (1972) Cancer Chemotherap. Reports *3*: 1-103.

2. Drug treatment and cytotoxicity assays. Cytotoxicity was assessed by determining the efffects of the various compounds upon either cell growth after 72 hr. of continous exposure, or colony formation after 5 days of continous exposure.

For the cell growth inhibition assays, 96-well microtiter plates were inoculated with 2 x 10^3 cells /well (10^4 cells/mL) and the cells were incubated at 37 °C overnight to permit attachment. The medium was then replaced with 180 µL/well of fresh growth medium, to which was added various compounds (20 µL of 10X stock in 100% dimethylsulfoxide (DMSO);
final concentration of DMSO being 0.2% which has no effect on cell growth). Cells were incubated for 72 hr in the presence or absence of drug, after which was added 50 µL of XTT/PMS solution prepared fresh by the following procedure: 8 mg XTT was dissolved in 100 µL DMSO; to this was added phosphate-buffered saline (PBS) without cations to a final XTT concentration of 2 mg/mL (final volume = 4 mL). 20 µL of PMS solution (3 mg/mL in PBS) was then added to the XTT solution. The cells were incubated with XTT/PMS for 90 min at 37°C, after which the A_{450} was determined by using a Thermomix ™ microplate reader (Molecular Devices). Growth inhibition was calculated from the values of drug treated and untreated by means of a computer program. A_{450} of wells containing growth medium and XTT solution only was treated as the null value.

For colony formation assays, AuxB1 or CH^RC5 cells were inoculated to 60 mm plastic cultures dished (300 cells/dish; cloning efficiency was approm. 70% for both lines) and incubated overnight to permit attachment. Stock solutions of the various compounds were added to the cells, and incubation was continued for 5 days to permit the development of macroscopic colonies were washed once with PBS and fixed with methanol. After staining with Giemsa, numbers of colonies/plate were determined with a Bioatran™ automatic counter (New Brunswick Scientific). Percent survival at each drug concentration was determined from the ratio of the number of colonies in the drug-treated sample divided by the number in the control (DMSO vehicle-treated) sample.

3. DNA Strand Break Assay. DNA single strand breaks produced in AuxB1 and CH^RC5 cells by the camptothecin analogues and amsacrine were assayed by alkaline elution. Mattern, et al., (1987) Cancer Res. *47*: 1793-1798. Cells were inoculated into 60-mm plastic tissue culture dishes (4 x 10^5 cells/dish) and incubated for 16-20 hr in medium containing 0.04 μCi/mL [^{14}C]-methyl thymidine (50 mCi/mmol; DuPont/New England Nuclear Corp) to label the DNA uniformly. Internal standard cells were labelled for the same time period with 0.1 μCi/mL [^{3}H]-methyl-thymidine (80 Ci/mmol; DuPont/NEN). Medium containing radiolabel was then removed and replaced with 37°C medium containing no radiolabel; cells were incubated in the latter for 60 min. To the AuxB1 and CH^RC5 cells labelled with ^{14}C were added stock solutions of the various drugs (or DMSO); incubation was continued for an additional 2 hr, after which samples were processed for alkaline elution as described. *Id.* ^{3}H- and ^{14}C-labelled cells were γ-irradiated with a Gammacell 40™ cesium source (Atomic Energy of Canada Ltd; dose rate = 104 rads/min), the growth medium having been replaced with 4°C PBS. Cells were deposited onto nucleopore filters, and the lysis solution (0.1 M glycine-0.025 M disodium ethylenediammine-tetraacetic acid [Na_2EDTA] - 2% sodium dodecyl sulfate [SDS], pH 10) contained 0.5 mg/ml proteinase K (Sigma). DNA was eluted with tetrapropylammonium hydroxide, pH 12.1 (Aldrich Chemical Co.) DNA single strand break frequency was calculated and expressed as "rad equivalents" by the internal standard method. Kohn, et al., (1981) "Measurement of Strand Breaks and Cross-links by Alkaline Elution" *in* Friedberg et al., *eds.* "DNA Repair: A Laboratory Manual of Research Techniques" New York, Marcel Dekker, 379-341.

3. *In Vivo* Studies:

A. Adjuvant-induced arthritis in rats

Adjuvant-induced arthritis (AA) was produced in Lewis rats by a single intradermal injection of 0.75 milligrams of *Mycobacterium butyricum* in light paraffin oil, into the base of the tail. The adjuvant arthritis occurs after a delay of approximately 10 days and is characterized by inflammation of the hindpaws. In prophylactic studies, iso-aaptamine was administered daily for 5 days, beginning on the sixth day after adjuvant injection. Hindpaw volumes were measured plethysmographically on days 14, 17 and 20. Inhibition of AA was determined according to the following formula:

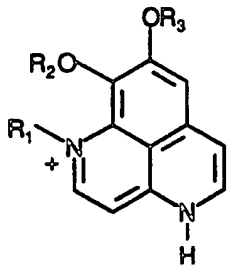
$$\% \text{ inhibition} = \frac{\left(\begin{array}{c}\text{paw volume in} \\ \text{arthritic control rats}\end{array} - \begin{array}{c}\text{paw volume in} \\ \text{drug treated rats}\end{array}\right)}{\left(\begin{array}{c}\text{paw volume in} \\ \text{arthritic control rats}\end{array} - \begin{array}{c}\text{paw volume in} \\ \text{nonarthritic control rats}\end{array}\right)} \times 100$$

Results

Daily intraperitoneal administration of iso-aaptamine to AA showed iso-aaptamine to be active at about 2 mg/kg.

What is claimed is:

1. A method of treatment of PKC-mediated disease states comprising administering to a mammal in need thereof a compound of Formula (I):



(I)

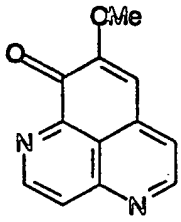
wherein:

R_1 is methyl or hydrogen,

R_2 is methyl, acetyl, benzoyl, or hydrogen, and

R_3 is methyl, acetyl, benzoyl or hydrogen;

a compound of Formula (II):



(II)

or a pharmaceutically acceptable salt thereof.

2. A method of treatment according to Claim 1 wherein said compound is a compound of Formula (I).

3. A method of treatment according to Claim 2 wherein R_1 is methyl, R_2 is hydrogen, and R_3 is methyl.

4. A method of treatment according to Claim 2 wherein R_2 is benzoyl.

5. A method of treatment according to Claim 4 wherein R_3 is bromobenzoyl.

6. A method of treatment according to Claim 1 wherein said PKC-mediated disease state is a cardiovascular disorder.

7. A method of treatment according to Claim 6 wherein the cardiovascular disorder is hypertension.

8. A method of treatment according to Claim 1 wherein said PKC-mediated disease state is inflammation.

9. A method of treatment according to Claim 8 wherein the inflammation is caused by arthritis.

10. A method of treatment according to Claim 1 wherein said PKC-mediated disease state is cancer.

11. A method of treatment according to Claim 10 wherein said compound is used as an adjuvant with an antineoplastic compound to ameliorate or prevent multiple drug resistance in the treatment of cancer with said antineoplastic compound.

12. A method of treatment according to Claim 1 wherein said PKC-mediated disease state is renal failure.

13. A method of treatment according to Claim 1 wherein said PKC-mediated disease state is septic shock.

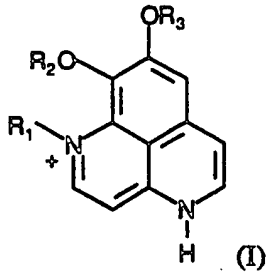
14. A method of treatment according to Claim 1 wherein said PKC-mediated disease state is an immunosuppressive disorder.

15. A method of treatment according to Claim 14 wherein said immunosuppressive disorder is AIDS.

16. A method of treatment according to Claim 1 wherein said PKC-mediated disease state is a central nervous system disorder.

17. A method of treatment according to Claim 1 wherein said PKC-mediated disease state is psoriasis.

18. A use of a compound of Formula (I):



(I)

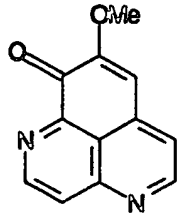
wherein:

R_1 is methyl or hydrogen,

R_2 is methyl, acetyl, benzoyl, or hydrogen, and

R_3 is methyl, acetyl, benzoyl or hydrogen;

a compound of Formula (II):



(II)

or a pharmaceutically acceptable salt thereof, for treatment of PKC-mediated disease states.

19. A use according to Claim 18 wherein said compound is a compound of Formula (I).

20. A use according to Claim 19 wherein R_1 is methyl, R_2 is hydrogen, R_3 is methyl.

21. A use according to Claim 19 wherein R_2 is benzoyl.

22. A use according to Claim 21 wherein R_3 is bromobenzoyl.

23. A use according to Claim 18 wherein said PKC-mediated disease state is a cardiovascular disorder.

24. A use according to Claim 23 wherein said cardiovascular disorder is hypertension.

25. A use according to Claim 18 wherein said PKC-mediated disease state is inflammation.

26. A use according to Claim 25 wherein said inflammation is caused by arthritis.

27. A use according to Claim 18 wherein said PKC-mediated disease state is cancer.

28. A use according to Claim 27 wherein said compound is used as adjuvant with an antineoplastic compound to ameliorate or prevent multiple drug resistance in the treatment of cancer with said antineoplastic compound.

29. A use according to Claim 18 wherein said PKC-mediated disease state is renal failure.

30. A use according to Claim 18 wherein said PKC-mediated disease state is septic shock.

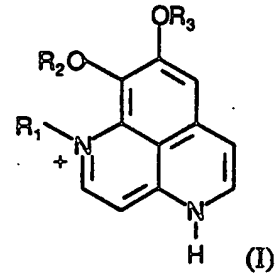
31. A use according to Claim 18 wherein said PKC-mediated disease state is an immunosuppressive disorder.

32. A use according to Claim 31 wherein said immunosuppressive disorder is AIDS.

33. A use according to Claim 18 wherein said PKC-mediated disease state is a central nervous system disorder.

34. A use according to Claim 18 wherein said PKC-mediated disease state is psoriasis.

35. A pharmaceutical composition comprising a compound of Formula (I):



(I)

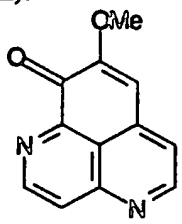
wherein:

R_1 is methyl or hydrogen,

R_2 is methyl, acetyl, benzoyl, or hydrogen, and

R_3 is methyl, acetyl, benzoyl or hydrogen;

or a compound of Formula (II):



(II)

or a pharmaceutically acceptable salt thereof,together with a pharmaceutically acceptable carrier, diluent or excipient.

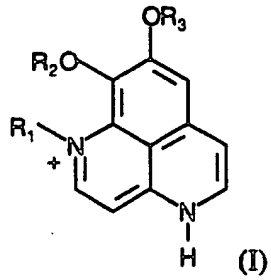
36. A pharmaceutical composition according to Claim 35 wherein said compound is a compound of Formula (I).

37. A pharmaceutical composition according to Claim 36 wherein R_2 is benzoyl.

38. A pharmaceutical composition according to Claim 35 wherein R_3 is bromobenzoyl.

39. A pharmaceutical composition according to Claim 35 wherein R_1 is methyl, R_2 is OH, and R_3 is methyl.

40. A compound of Formula I:



(I)

wherein:

R_1 is methyl;

R_2 is hydrogen; and

R_3 is methyl, acetyl, or benzoyl.

41. A compound according to Claim 40 wherein R_3 is methyl.

42. A compound according to Claim 40 wherein R_3 is benzoyl.

43. A compound according to Claim 42 wherein R_3 is bromobenzoyl.

44. A compound according to Claim 43wherein R_3 is 4-bromobenzoyl.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/09497

A. CLASSIFICATION OF SUBJECT MATTER
IPC(5) :A61K 31/44; C07D 471/00
US CL :546/81; 514/292
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 546/81; 514/292

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J. Chem. Soc. Perkin Trans 1, issued 1987, Hideshi Nakamura et al. "Aaptamines. Novel Benzo[de][1,6]naphthyridines from the Okinawan Marine Sponge Aaptos aaptos", pages 173-176, see structures 1, 2 and 3.	35,36,40 and 41
X	Journal of Natural Products, Volume 56, No. 6, issued June 1993, Ross E. Longley et al, "Evaluation of Marine Sponge Metabolites For Cytotoxicity And Signal Transduction Activity", pages 915-920, see entire document.	35,36,40 and 41

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document published on or after the international filing date
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

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